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ATM plays antioxidant, boosting mitophagy *via* denitrosylation

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ABSTRACT

Mitophagy is a selective process aimed at removing damaged or burned-out mitochondria; it is activated upon different stimuli and plays a fundamental role in preventing overproduction of reactive oxygen species (ROS) that might be generated by dysfunctional mitochondria. From this angle, mitophagy can be considered a fully-fledged antioxidant process. Such a surrogate antioxidant function is recently emerging, being shared among many molecular pathways and players that are usually not included among – and, formally, do not directly act as – antioxidants. ATM (ataxia telangiectasia mutated) is a prototype of this class of "neglected" antioxidants. In spite of its well-known role in DNA damage response, many phenotypes of ataxia telangiectasia (A-T) patients are, indeed, related to chronic oxidative stress, arguing for an additional antioxidant role of ATM. In a recent study, we discovered the mechanism through which ATM exerts antioxidant activity. In particular, we provided evidence that this involves ADH5/GSNOR (alcohol dehydrogenase 5 (class III), chi polypeptide), which, in turn, sustains mitophagy via PARK2 denitrosylation, and protects the cell from detrimental effects due to ROS.

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Besides acting as an early sensor of DNA damage, in the last decade ATM (ataxia telangiectasia mutated) has been frequently proposed to play additional roles in the cell response to stress, most of which are postulated on the basis of the phenotypes observed in $atm^{-/-}$ mice. Among these new additional functions, the hypothesis that ATM has antioxidant activity is gaining increasing relevance. The discovery that ATM can auto-phosphorylate and activate in response to pro-oxidant stimuli *via* disulfide-mediated dimerization has given further support to the idea that ATM represents a paradigmatic redox-sensitive kinase. However, despite the fact that this alternative mechanism – which takes place even in the absence of DNA damage – has been elucidated, how this confers to ATM the ability to act as an antioxidant is still unknown.

Recent results indicate that this oxidized active dimer of ATM triggers pexophagy in response to hydrogen peroxide through the phosphorylation of PEX5/PTS1R (peroxisomal biogenesis factor 5). Peroxisome numbers and homeostasis need to be properly maintained as these organelles contain oxidases that produce hydrogen peroxide as a reaction (by) product that can be harmful to the cell. From this point of view, pexophagy might represent a likely prototype mechanism through which ATM prevents oxidative stress.

In our work, we provide evidence that ATM activation by hydrogen peroxide is also required to sustain mitophagy [1], thus extending the antioxidant relevance of ATM as a selective autophagy regulator of all those organelles that are endogenous sources of reactive oxygen species (ROS). In particular, we demonstrated that ATM sustains mitophagy through the upregulation of the denitrosylase ADH5/GSNOR, whose activity has been previously found crucial in mitochondrial homeostasis (Figure 1). Pharmacological inhibition and a reverse genetics approach, using siRNA against *Atm*, *Chk2* (checkpoint kinase 2) and *Trp53/p53* (the mouse gene is *Trp53*, but we use TP53 to refer to the protein hereafter for simplicity), indicated that all these factors – which belong to the same signaling axis (ATM-CHK2-TP53) – are required to enhance the ADH5 translation rate. Interestingly, *Adh5* mRNA shows two untranslated open reading frames (uORFs) at the 5′ UTR that are recognized by ribosomes, but are not proficient to initiate translation. This results in ribosome stalling, which keeps the ribosome far from the transcription start sequence and slow the rate of translation. It is recently emerging that TP53 directly regulates translational efficiency of specific sets of mRNAs that are activated upon stress (such as the uORF-containing genes). Although still not defined, it is reasonable that TP53 modulates *Adh5* mRNA translation in the same way.

ADH5 catalyzes protein denitrosylation and, in doing so, sustains mitophagy. Consistently, we observed that hydrogen peroxide results in a reduction of the levels of *S*-nitrosylated (SNO)-proteins, that is reverted by the pharmacological inhibition of ATM. Moreover, transfections with siRNAs against *Adh5* and *Atm*, as well as the expression of the ATM redox-insensitive mutant (ATM^{C2991L}), are associated with mitophagy defects, which are rescued by ADH5 ectopic expression. Conversely, cells expressing the wild-type and the DNA

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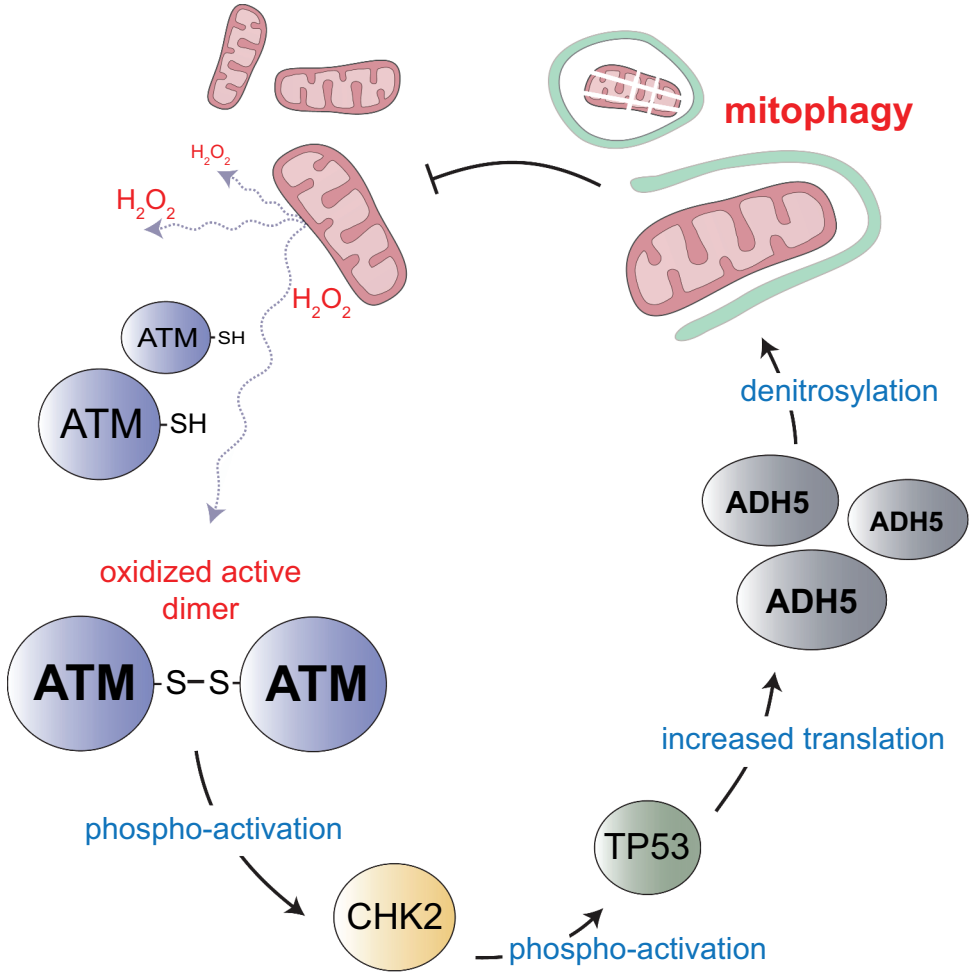

Figure 1. ATM is activated by hydrogen peroxide (H_2O_2) – e.g., when produced by dysfunctional mitochondria – by a (non-canonical) redox mechanism that involves the formation of a disulfide bridge linking C2991 of two ATM protomers. In this dimeric activated form, ATM phosphorylates and activates CHK2, which in turn activates TP53 to enhance *Adh5* mRNA translation. This signaling pathways is required to increase the expression of ADH5 that, by denitrosylating proteins involved in mitophagy (i.e., PRKN/PARK2, not shown in the scheme) boosts mitochondrial removal to eliminate the source of oxidative stress and protect the cells from death. We demonstrated that this mechanism is particularly relevant to guard from detrimental side effects deriving from simultaneous fluxes of nitric oxide and hydrogen peroxide, e.g., during T cell activation.

damage-unresponsive form of ATM (ATM[2RA]) are able to correctly recognize and remove mitochondria in cells subjected to treatment with hydrogen peroxide or ROS generators. This aspect is worth emphasizing as the ATM-ADH5 axis is not involved in mitophagy induced by other stimuli that are not associated with ROS production (e.g., hypoxia or antimycin-oligomycin treatment).

Altogether, these results support the hypothesis that the ATM-ADH5 axis keeps mitophagy at full capacity by maximizing protein denitrosylation, and confirm previous evidence arguing for the two activation mechanisms of ATM (in response to DNA damage or oxidants) being separated and independent.

Our study indicates that ADH5 has a double role: it detoxifies from SNO adducts and prevents mitochondrial ROS production by sustaining the mitophagy rate. Therefore, it is reasonable to hypothesize that ADH5 activity extends beyond denitrosylation, and produces wider protective effects against nitroxidative conditions, such as those arising from simultaneous fluxes of nitric oxide (NO) and hydrogen peroxide. Consistent with this view, we observed that ADH5 ablation makes cells vulnerable to the combined treatment with hydrogen peroxide and the NO donor dipropylenetriamine/DPTA NONOate, whereas ADH5-overexpression confers resistance to cell death; a phenomenon that is even more pronounced if ATM is pharmacologically inhibited.

Among cells facing and adapting to constant NO and hydrogen peroxide fluxes, immune cells (namely, T lymphocytes) deserve to be mentioned. Indeed, in response to host attack, or upon tumor cell recognitions, NOS2/inducible NO synthase and NADPH oxidases are contextually activated, this event being crucial for T-cell activation and clonal expansion. Interestingly, besides severe impairment of locomotor activity and genome integrity maintenance, the main pathological phenotypes typifying A-T patients is a widespread immune deficiency and lymphopenia, which is largely ascribed to a decreased number of naïve $CD4^+$ cells that die by excessive apoptosis in the thymus. This defect exposes A-T patients to severe infections which, basically, represent the major cause of death associated with A-T. Strikingly, *adh5*$^{-/-}$ null mice entirely phenocopy this immunological condition (i.e., a systemic reduction of naïve $CD4^+$ T cells), supporting the functional relationship between ATM and ADH5, and providing the

biological relevance of this signaling pathway. Consistent with this, experiments in which we activated, *in vitro*, Jurkat cells and human $CD4^+$ T lymphocytes show a significant increase of apoptosis if ADH5 or ATM are pharmacologically inhibited. This condition is accompanied by a reduction of proliferating (activated) blasts, confirming that the ATM-ADH5 signaling axis is required to prevent detrimental side effects of endogenous NO and hydrogen peroxide (over)production.

Overall, our results add a new piece in the complex puzzle of ATM roles and the manifold ways through which it is involved in cellular response to stress. In particular, our study offers a molecular rationale in support of previous observations pointing to mitochondrial defects in A-T lymphocytes. Last, but not least, our data suggest that other diseases related to ATM dysfunction or mutation (e.g., breast cancer) might derive from a reduced denitrosylating capacity. In support of this hypothesis, *N*-acetyl-L-cysteine/NAC – which is an efficient denitrosylating molecule – has been demonstrated to be beneficial in $atm^{-/-}$ mice, and is currently in phase 2 clinical trials to validate any favorable effects in A-T patients.

Disclosure statement

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Reference

[1] Cirotti C, Rizza S, Giglio P, et al. Redox activation of ATM enhances GSNOR translation to sustain mitophagy and tolerance to oxidative stress. EMBO Rep. 2020 Nov;26:e50500.